CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA[1]
NICOTINE

Chemical Code # 075, Tolerance # 51983
SB 950 # 768
Original date: January 3, 2003

I. DATA GAP STATUS

Chronic/Onco toxicity, rat:	Data gap, inadequate studies, no adverse effect indicted.
Chronic toxicity, dog:	Data gap, no study on file
Oncogenicity, mouse:	Data gap, inadequate study, no adverse effect indicated.
Reproduction, rat:	Data gap, inadequate studies, possible adverse effects indicated
Teratology, rat:	Data gap, inadequate studies, possible adverse effects indicated.
Teratology, rabbit:	Data gap, no study submitted.
Teratology, mouse:	Data gap, inadequate study, possible adverse effects indicated.
Gene mutation:	Data gap, inadequate studies, no adverse effect indicated.
Chromosome effects:	Data gap, inadequate studies, possible adverse effects indicated.
DNA damage:	Data gap, inadequate studies, possible adverse effects indicated.
Neurotoxicity:	Not required at this time.

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[1] Studies are Journal articles: The articles provide useful information, but lack necessary information and data for acceptable studies.
Toxicology one-liners are attached.
All record numbers through 135213 were examined.
** Indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T030103; compiled by: J. Kishiyama and J. Gee, January 3, 2003

There are currently two products registered in California, both with signal word of "danger".

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. No individual worksheets have been prepared for these publications. Volume 51983-001 was received in 1995 and contained selected publications compiled by the registrant. A search of the recent open literature indicates that there are numerous additional publications addressing the toxicity of nicotine, especially with regard to the nervous system and developmental/reproductive effects. These recent publications have not been reviewed for this Summary of Toxicology Data. Altogether, the hazards of nicotine (and tobacco smoke) have been well substantiated in humans as well as in laboratory animal studies. In addition, nicotine is available OTC as a "drug" for human use to stop smoking. (Gee, 1/3/03)

001 135214 Ames, B. N. and L. S. Gold "Chemical carcinogenesis: Too many rodent carcinogens." (Publ. in *Proc. Nat. Acad. Sci.* 87: 7772 - 7776 (1990)) The authors discuss testing at the maximum tolerated dose (MTD), which stimulates mitogenesis, thereby increasing mutagenesis and carcinogenesis. They note that about half of the chemicals tested in rodent bioassays, whether synthetic or natural, have been found carcinogenic at the MTD. No worksheet. Supplemental publication. (Gee, 1/2/03).

COMBINED, RAT

001 135210 Thompson, J. H., F. D. Irwin, S. Kanematsu, K. Seraydarian, and M. Suh. "Effects of Chronic Nicotine Administration and Age in Male Fisher 344 Rats." (Publ. in *Toxicology and Applied Pharmacol*ogy 26: 606-620 (1973)). Nicotine was administered by subcutaneous injection at a dose of 0 (physiologic saline) or 1000 μg base/ml/kg/day (in 6% gelatin) to male Fischer 344 rats for 2 (control n = 6, treated n = 6) or 22 months (control n = 10, treated n = 38). Four control and 10 treated rats died in the 22-month groups due to food and water deprivation over a weekend. Bodyweight gain was reduced for nicotine treated rats. Hematological evaluations revealed no significant changes. There was no affect of treatment on Ca-dependent myosin ATPase or lactic acid dehydrogenase activity. Mg-dependent ATPase activity of soleus muscle myofibrils was depressed at 22 months in treated rats [statistical analysis was not possible as samples were pooled]. Neoplasms were reported for both old control and treated animals. Leydig cell hyperplasia of the testes, common in older rats, occurred at an incidence of 89% (34/38) for nicotine treated animals compared to 66% (4/6) in controls and was statistically significant ($p < 0.05$). The reason for this significant difference was unclear but the authors suggest premature aging may have been caused by nicotine treatment as a possible explanation. Adverse effects not identifiable from data due to inadequate number of animals at risk. No worksheet. (Kishiyama and Gee, 12/31/02).

001 135212 LaVoie, E. J., A. Shigematsu, A. Rivenson, B. Mu and D. Hoffmann. "Evaluation of the Effects of Cotinine and Nicotine-N'-Oxides on the Development of Tumors in Rats Initiated with N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide." (Naylor Dana Institute for Disease Prevention, publ. in *J. National Cancer Inst*. 75 (6): 1075-1081 (December, 1985)) N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT), at 0.1% in agar as the initiating agent, was fed for 6 weeks to groups of male F344 rats. After completion of FANFT treatment, the rats received one of four treatments in drinking water, weeks 18 - 96: cotinine (99% pure), *trans*-nicotine-N'-oxide alone or a mixture of *cis*- and *trans* nicotine-N'-oxide (36:64), at concentrations of 0.1, 0.02, and 0.02%, respectively, for the next 78 weeks (to week 96 of age). Tissues from the urinary

bladder, prostate, testes, thyroid, pancreas, esophagus, tongue and trachea were examined histologically as were gross lesions. RESULTS: The average weight gain in the FANFT group was lower than controls, being 136.6 g compared with 168.5 g/rat. During 6 weeks on control diet and tap water, treated animals made substantial gains. Rats receiving either *trans*-nicotine N'-oxide or the mixture consumed less water than either controls or cotinine group. Cotinine did not affect the incidence of urinary bladder transitional cell tumors. The *cis* and *trans* mixture and *trans*-nicotine N'-oxides actually decreased the incidence. Tumor formation in the tongue and palate with FANFT was not affected by any subsequent treatment. There was, however, a significant increase in forestomach tumors initiated with FANFT with exposure to the *trans* and *cis* isomers of nicotine-N'-oxide. Cotinine and the isomers of nicotine-N'-oxide were not tumorigenic in rats fed control diets. Supplemental study. No worksheet. (Kishiyama and Gee, 12/31/02).

CHRONIC TOXICITY, DOG

No Study Submitted.

ONCOGENICITY, MOUSE

001 135211 Toth, B. "Effects of Long Term Administration of Nicotine Hydrochloride and Nicotinic Acid in Mice." (The Eppley Research in Cancer and Allied Diseases, publ. in *Anticancer Research* 2: 71-74 (1982)). Solutions of nicotine hydrochloride at 0% (untreated, 100/sex), 0.09375% and 0.0625% and nicotinic acid at 1% were administered in the drinking water during the lifetime to 50 Swiss albino mice/sex/treatment group. Estimated daily intakes were 4.3 mg and 5.3 mg for females and males at 0.09375% nicotine, 4.0 and 5.1 mg for females and males at 0.0625% nicotine HCl and 81 and 107 for females and males, nicotinic acid. Histological examinations were made for selected tissues and gross changes. There was no affect on survival with animals continued until death or killed in poor condition (week 140 for last survivor). There was no evidence of oncogenicity. Supplemental study. No worksheet. No adverse effect reported. (Kishiyama and Gee, 12/31/02).

REPRODUCTION, RAT

001 135195 Hudson, D. B. and P. S. Timiras. "Nicotine Injection during Gestation: Impairment of Reproduction, Fetal Viability, and Development." (Department of Physiology-Anatomy, U.C. Berkeley, CA, publ. in: *Biology of Reproduction* **7:** 247-253 (1972)) Nicotine was injected subcutaneously twice daily at doses of 0 (0.9% saline), 1, 3, and 5 mg/kg per injection (total daily doses of 2, 6 and 10 mg/kg) to mated female Long-Evans rats. The number per group varied (from 4 to 13). There were two series of treatments. Series I were treated from day 0 to day 21 and terminated in all animals when the first one delivered. Series II were injected with saline or 3 mg/kg, days 0 - 7 of gestation and allowed to deliver spontaneously. Maternal weight gain, length of pregnancy, and birth weight of offspring were determined for those delivering. By C-section, the number of fetuses, crown-rump length, placental weight and number of corpora lutea were recorded. Nicotine effects were similar for both series: a dose related decline in reproductive capacity and an increase in resorptions were observed with increasing dose. Maternal mortality was increased (36% and 38% at 3 and 5 mg/kg, respectively) and the number of viable litters decreased, body weight of fetuses by C-section and maternal body weight gain were reduced in the mid and high dose groups. Newborn body weights were comparable but

gestation length was statistically significantly longer at 3 and 5 mg/kg/dose. The percentage of implants was not affected but fewer survived. Possible adverse effect on reproduction. Supplemental study. No worksheet. Note: the dose of 1 mg/kg (2 mg/kg daily total) was stated to be within the range for a heavy smoker. (Kishiyama and Gee, 12/20/02).

001 135196 Riesenfeld, A. and H. Oliva. "The Effect of Nicotine and Alcohol on the Fertility and Life Span of Rats, a Cytological Analysis." (Publ. in *Acta anat*. 128: 45-50 (1987)). Nicotine was administered by intramuscular injection at a dose of 0.42 mg/kg, three times daily, with the injection sites rotated, for 90 days (interrupted for 30 days during nursing). Dosing was initiated at 50 days of age, before the onset of puberty with female Fisher rats (96) and female Buffalo rats (46). Of these, 50% of the Fisher and 49.7% of the Buffalo rats were sterile, compared with a historical rate of 21%. The life span of both nicotine treated maternal rat species was significantly shortened (110 days versus 502 for Fisher and 131 versus 472 for Buffalo rats). The age at first parturition was comparable for Fisher rats (84 versus 86 for controls) but the last parturition was statistically lower (175*** versus 386 days of age). For Buffalo rats, the first parturition was delayed on average by 21 days (105.4 versus 84.5 for controls) and the last was also earlier (175 versus 465 for controls). The number of neonates for Fisher rats was 76 for 48 injected dams and 21 for Buffalo rats. Offspring of treated dams that were fetally and postnatally exposed to nicotine either failed to give birth or had offspring that died shortly after birth, becoming "extinct" after one generation. Cytology revealed a mild increase in lymphocytes and/or polymorphonuclear leukocytes in nicotine treated rats, appearing much earlier in Buffalo than Fisher rats and considered associated with inflammation. Offspring (untreated themselves) were normal as were offspring born after treatment of dams ceased, the inflammation seeming to be reversible. Supplemental study with a possible adverse effect. No worksheet. (Kishiyama and Gee, 12/20/02).

001 135967 Hammer, R. E. and J. A. Mitchell. "Nicotine Reduces Embryo Growth, Delays Implantation and Retards Parturition in Rats." (Dept. of Anatomy, School of Medicine, Wayne State Univ, Detroit, MI, publ. in *Proceedings of The Society for Experimental Biology and Medicine* 162: 333-336 (1979) Nicotine, 98% pure, was injected subcutaneously at a dose of 5 mg/kg (twice daily) during postcoitum days 0 through 5 to mated female Sprague-Dawley rats. Two test series were used, one to evaluate embryo growth and the other to determine effects of nicotine on fecundity and time of parturition. For effects on implantation, uterine horns were flushed with saline at selected times and the blastocysts retrieved. Embryos could be retrieved longer in nicotine exposed dams, indicating a longer time to implantation. Loss of the zona pellucida was slower and cell proliferation was significantly reduced in embryos of nicotine treated rats. Delivery time was significantly delayed for nicotine-treated rats, but the size, weight, sex, or mortality of offspring was not significantly affected. Supplemental study. No worksheet. (Kishiyama and Gee, 12/20/02).

001 135198 Hamosh, M., M. R. Simon, and P. Hamosh. "Effect of Nicotine on the Development of Fetal and Suckling Rats." (Department of Physiology and Biophysics, Georgetown Univ. Med. School, publ. in *Biol. Neonate* 35: 290-297 (1979)). Nicotine (98-100% pure) was administered either by subcutaneous injection (3 x daily: 1/3 of total at each time at a different site) and/or osmotic minipump (to alleviate the stress of injections) at doses of 0 (untreated), 0 (sterile saline by injection), 100 μg/kg/day or 1 mg/kg/day to mated female Sprague-Dawley rats, 6 - 11 per group. Dosing began on day 14 of gestation and continued throughout the study. Fetuses were collected on day 20 of gestation and weight, length measured. Others were allowed to deliver normally. Suckling rats were sacrificed at several ages for examination of length, weight and stomach contents for lipid content and free fatty acids. Litter

size was reduced for the higher dose group, being 8.8 versus 10.0 in controls, with 6 stillborn to 5 dams versus 1/12 litters in controls. The development of pups from nicotine treated dams (100 ug/kg/day) appeared normal at birth and up to one week after birth, but thereafter became slower in terms of weight and length. Stomach contents were smaller in pups of nicotine-treated dams. Fat content in mg was lower at day 12 in pups of treated dams (100 ug/kg/day) and lipolytic activity was slower to increase, reaching normal levels by day 7. Nicotine interference with milk production was suggested. Supplemental study. No worksheet. (Kishiyama and Gee, 12/20/02).

001 135199 Daeninck, P. J., N. Messiha, and T. V. N. Persaud. "Intrauterine Development in the Rat following Continuous Exposure to Nicotine from Gestational Day 6 through 12." (Department of Anatomy, University of Manitoba, publ. in *Anat. Anz. Jena* 172 : 257-261 (1991)). Nicotine was administered to 10 mated Sprague-Dawley female rats per group via osmotic minipump, implanted subcutaneously, at doses of 0 (saline), 75 and 150 μg/hour during gestation days 6 through 12 (7 days). The uterine horns were examined on Day 12. Embryo development (yolk sac diameter, crown-rump length, head length, olfactory system, and the otic and optic systems) was delayed significantly with nicotine at both 75 and 150 μg/hour. Additional developmental endpoints were affected at 150 μg/hr (including lower values for hindbrain, midbrain, forebrain, and heart). Supplemental study. No worksheet. Possible adverse effects on development. (Kishiyama and Gee, 12/30/02).

TERATOLOGY, RAT

001 135201 Joschko, M. A., I. E. Dreosti, and R. S. Tulsi. "The Teratogenic Effects of Nicotine *In Vitro* in Rats: A Light and Electron Microscope Study." (Publ in *Neurotoxiclogy and Teratology* 13: 307-316 (1991)). Embryos were dissected on day 9.5 from pregnant Sprague-Dawley rats. Nicotine (98-100%, free base) at concentrations of 0, 100, 200, 300 and 400 μg/ml was added *in vitro* to 14-21 explanted embryos/group. After 48 hours in culture, the embryos were examined for impaired growth and gross dysmorphology. Four control and 5/treatment group were prepared for light and electron microscopy. Embryo growth retardation (crown-rump length, yolk sac diameter and reduced somite number) was observed in a dose dependent manner, although not all parameters were statistically significant at 100 μg/ml. Neural tube dysmorphology was noted in 20% of the fetuses at 100 μg/ml and nearly all fetuses at 300 μg/ml. Branchial arch defects, heart defects, absence of forelimb buds, and eye defects increased with concentration. Cell disruption and necrosis in the neuroepithelium and mesenchyme were reported using EM. Supplemental study. No worksheet. Possible adverse effects on development. (Kishiyama and Gee, 12/30/02).

TERATOLOGY, MOUSE

001 135200 Yousef, A., L. P. Gartner, and J. L. Hiatt. "Teratogenic Effects of Nicotine on Palate Formation in Mice." (Department of Anatomy Dental School, University of Maryland, publ. in *Biological Structures and Morphogenesis* 3 (1): 31-35 (1990-1991)) Nicotine sulfate was injected intraperitoneally at doses of 0 (0.1% sterile physiological saline and non-injected) and 1.67 mg/kg body weight/day, from day 6 through 15 of gestation to CD-1 Swiss albino mice, 18 - 22 per group. Mice were sacrificed on day 18 of gestation and the fetuses evaluated for gross and histological changes. Fetal viability, crown-rump length, weight, and head dimensions were determined. The heads of the first two fetuses per litter were used for gross morphological

examination of the palate. The remainder were fixed and embedded for histological evaluation. Maternal bodyweight gain was reduced for nicotine treated mice (14.4 grams vs. 22.7 grams). Fetuses from nicotine treated mice had lower crown-rump length (15.1 mm versus 21.6 for saline control and 24.6 for uninjected control), weighed less, had lower head width, height and circumference. Fetuses from treated dams had a higher incidence of incompletely fused palates (9.6% (16/166) versus none in either control group (N = 430). Histological examination showed that in most of the fetuses with cleft palate, the palatal shelves were in the vertical position with the tongue interposed between the shelves. Supplemental study. No worksheet. Possible adverse developmental effects (cleft palate, growth retardation). (Kishiyama and Gee, 12/30/02).

TERATOLOGY, RABBIT

No Study Submitted.

GENE MUTATION

001 135202 Riebe, M., K. Westphal, and P. Fortnagel "Mutagenicity Testing, in Bacterial Test Systems, of Some Constituents of Tobacco." (Publ. in *Mutation Research* 101: 39-43 (1982)) Twelve constituents of tobacco and a range of concentrations were tested in the Ames assay with *Salmonella typhimurium* and with *Escherichia coli pol*A^+/*pol*A^- strains. Concentrations were as follows: Nicotine (1 - 20 mM), nicotine 1'-N-oxide (0.15 - 3 mM), cotinine (0.25 - 5 mM), myosmine (1.5 - 30 mM), nicotyrine (0.16 - 3.2 mM), anabasine (0.5 - 10 mM), anatabine (0.15 - 3 mM), 2,3'-dipyridyle (1.75 - 35 mm), pyrrole (1.75 - 35 mM), pyrrolidine (1.25 - 25 mM), piperidine (1.25 - 25 mM), and pyridine (1.6 - 32 mM). *Salmonella* strains were TA98, TA100 and TA1537, tested with and without activation using a preincubation of 1 hour before plating. There were three trials with triplicate plates. With *E. coli*, the diameter of growth inhibition using the spot test (in triplicate, two trials) and survival in liquid culture were determined. The twelve tobacco constituents did not significantly induce increases in revertants in the *Salmonella* strains. Unacceptable (missing details, strains). No adverse effects with *Salmonella*. See below for the results with *E. coli.* No worksheet. (Kishiyama and Gee, 12/30/02).

001 135203 Florin, I., L. Rutberg, M. Curvall, and C. R. Enzell "Screening of Tobacco Smoke Constituents for Mutagenicity Using the Ames' Test." (Publ. in *Toxicology* 18: 219 - 232 (1980)) Two hundred thirty nine (239) compounds, considered representative constituents of the gaseous or semivolatile phases of tobacco smoke, were evaluated for mutagenicity. *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 were used with and without metabolic activation (S9 from rat liver). A less sensitive spot testing was used to facilitate the testing of large numbers of compounds. Nicotine was negative. Nine compounds were, however, positive (5 hydrocarbons, 2 N-heterocycles and 2 amines). No adverse effect with nicotine. No worksheet. Supplemental study. (Kishiyama and Gee, 12/30/02)

CHROMOSOME EFFECTS

001 135204 Trivedi, A. H., B. J. Dave and S. G. Adhvaryu. "Assessment of Genotoxicity of Nicotine Employing *In vitro* Mammalian Test System." (Publ. in *Cancer Letters* 54: 89 - 94 (1990)) Nicotine (free base) was tested with Chinese hamster ovary cells at concentrations of

625 and 1000 μg/ml (first experiment) and at concentrations of 150, 250, 375, 500 and 625 μg/ml (second experiment) for genotoxicity without activation. Both chromosomal aberrations and sister chromatid exchanges were evaluated. In the first experiment, cells were exposed for 2 or 4 hours, washed, and BrdU added. For aberrations, cells were harvested after 24 hours and after 2 cell cycles for SCEs. In experiment 2, the nicotine was present until harvest at 24 (aberrations) or 48 hours (SCE), also with BrdU. For aberrations, 100 metaphases in MI were scored and 25 metaphases in MII for SCE. After 2 or 4 hours (experiment I), aberrations were increased at 1000 μg/ml, both exposure lengths. SCEs were statistically increased for both exposure times and concentrations. In experiment II, nicotine induced chromosome aberrations at concentrations of 375 μg/ml and higher with continuous exposure and increased SCE frequency at all tested concentrations (150-625 μg/ml). Possible adverse effects**.** No worksheet. Supplemental study (no activation). (Kishiyama and Gee, 12/30/02).

001 135205 Trivedi, A. H., B. J. Dave and S. G. Adhvaryu. "Genotoxic Effects of Nicotine in Combination with Arecoline on CHO Cells." (Publ. in *Cancer Letters* 74: 105 - 110 (1993)) Continuous and short-term (4 hour) nicotine and arecoline treatment were evaluated for genotoxicity (chromosome aberrations and sister chromatid exchange (SCE)). Continuous treatment consisted of nicotine (150 μg/ml) and arecoline (12.5 μg/ml) alone and also nicotine (150 μg/ml) with arecoline hydrobromide (2, 5, or 12.5 μg/ml) combinations, in the presence of BrdU. Cultures were incubated for 24 hours for aberrations and 48 hours (two cell cycles) for SCE. Short-term or pulse treatment (4 hours) consisted of nicotine at 300 μg/ml and 90, 200 or 300 μg/ml nicotine in combination with arecoline 50, 100, and 150 μg/ml, respectively. Following the pulse exposure, cultures were incubated for one cell cycle for aberrations or two cycles for SCE. No activation was included. At least 100 metaphases in MI were analyzed for aberrations and 25 in MII for SCE. With continuous treatment, 150 μg/ml nicotine did not increase aberrations but did result in a statistically significant increase in SCE. Pulse treatment with 300 μg/ml nicotine significantly increased SCE/cell. Combinations of nicotine and arecoline increased genotoxicity more than either chemical alone. Possible adverse effect (increased SCE with nicotine alone) Supplemental study. No worksheet. (Kishiyama and Gee, 12/30/02).

001 135206 Bishun, N. P., N. Lloyd, R. W. Raven and D. C. Williams. "The *In vitro* and *In Vivo* Cytogenetic Effects of Nicotine." (Publ. in *Acta Biologica Academiae Scientiarum Hungaricae* 23(2): 175-180 (1972)) Nicotine was evaluated for genotoxicity *in vitro* using human peripheral blood lymphocytes at concentrations of 0, 0.5, 1.0, 1.5, and 2.0 μg/ml with exposure starting when cultures were initiated. Incubation times were 6, 24, 48 and 72 hours with a total incubation time of 72 hours for all treatments. Duplicate cultures. I*n vivo*, groups of 12 randomly bred mice, ages from 5 weeks to 4 months, were injected with doses of 0 (saline), 0.07, 0.08, and 0.09 μg/total body weight, two injections/week for three weeks before sacrifice. Bone marrow cells were analyzed for aberrations. Cytotoxicity precluded genotoxic evaluation in the *in vitro* test**.** Aneuploidy and translocations were observed in vivo. Supplemental study. No worksheet. Possible adverse effect. (Kishiyama and Gee, 12/30/02).

001 135207 Riebe, M. and K. Westphal. "Studies on the Induction of Sister-Chromatid Exchanges in Chinese Hamster Ovary Cells by Various Tobacco Alkaloids." (Publ. in *Mutation Research* 124: 281 - 286 (1983)) Five tobacco alkaloids (nicotine, myosmine, anabasine, anatabine, and nornicotine) were tested with and without metabolic activation (rat liver) for genotoxicity using Chinese hamster ovary (CHO) cells. Cell cultures were treated for 1 hour, BrdUrd added and incubation continued for 42 hours. The assay was repeated 3 times with each alkaloid tested at three concentrations. Between 30 and 140 metaphases per concentration were

scored for SCE. PBS and DMSO were negative controls. Anatabine (0, 125, 250 or 500 μg/ml without S9 Mix) significantly increased the frequency of SCEs with increasing concentration; Nicotine (0, 1250, 2500 or 5000 μg/ml) and Nornicotine (0, 625, 1250 or 2500 μg/ml) without S9 activation slightly increased the frequency of SCEs**.** Anabasine and Myosmine (both at 0, 62.5, 125 or 250, with and without S9 Mix) did not increase the frequency of SCEs. Possible adverse effect. Supplemental study. No worksheet. (Kishiyama and Gee, 12/30/02).

DNA DAMAGE

001 135202 Riebe, M., K. Westphal, and P. Fortnagel "Mutagenicity Testing, in Bacterial Test Systems, of Some Constituents of Tobacco." (Publ. in *Mutation Research* 101: 39-43 (1982)) Twelve constituents of tobacco and a range of concentrations were tested in the Ames assay with *Salmonella typhimurium* and with *Escherichia coli pol*A^+/*pol*A^- strains. Concentrations were as follows: Nicotine (1 - 20 mM), nicotine 1'-N-oxide (0.15 - 3 mM), cotinine (0.25 - 5 mM), myosmine (1.5 - 30 mM), nicotyrine (0.16 - 3.2 mM), anabasine (0.5 - 10 mM), anatabine (0.15 - 3 mM), 2,3'-dipyridyle (1.75 - 35 mm), pyrrole (1.75 - 35 mM), pyrrolidine (1.25 - 25 mM), piperidine (1.25 - 25 mM), and pyridine (1.6 - 32 mM). *Salmonella* strains were TA98, TA100 and TA1537, tested with and without activation using a preincubation of 1 hour before plating. There were three trials with triplicate plates. With *E. coli*, the diameter of growth inhibition using the spot test (in triplicate, two trials) and survival in liquid culture were determined. The twelve tobacco constituents did not significantly induce increases in revertants in the *Salmonella* strains. Nicotine was one of several chemicals positive with *E. coli*, giving a larger diameter of growth inhibition with pol A^- strain. Unacceptable (missing details.) Possible adverse effect. No worksheet. (Kishiyama and Gee, 12/30/02)

001 135208 McFarland, B. J., F. J. Seidler and T. A. Slotkin. "Inhibition of DNA Synthesis in Neonatal Rat Brain Regions Caused by Acute Nicotine Administration." (Publ. in *Developmental Brain Research* 58: 223-229 (1991)) Nicotine dibitartrate was injected subcutaneously at a dose of 3 mg/kg nicotine (9 mg/kg as dibitartrate) to Sprague-Dawley rat pups at 1, 3, 8, 10 and 15 days of age. Immediately after nicotine injection, some pups were given [^{3}H]-thymidine and sacrificed after 30 minutes. Other groups of pups were injected with radiolabel at 30 min, 2 or 4 hours after nicotine injection with sacrifice 30 minutes later. Other pups were given [^{3}H]-leucine to measure protein synthesis at day 10 of age. Pups were sacrificed and brains dissected into three regions: midbrain + brainstem, cerebral cortex and cerebellum. Incorporation of total thymidine and thymidine into DNA were determined. To examine autonomic blocking drugs, pups were given s.c. injections of phenoxybenzamine HCl, chlorisondamine chloride or a mixture of propranolol HCl and atropine sulfate 15 minutes before the nicotine treatment. These drugs have been demonstrated to produce α-adrenergic, nicotinic ganglionic, β-adrenergic and muscarinic antagonism, respectively, in neonates. The role of hypoxia on nicotine was examined by ventilating cages with 100% oxygen during the 30 minute period after nicotine injection. Intracisternal injections were equivalent to 2 μg of nicotine in 10 μl. Incorporation of radiolabel was expressed as the ratio of the TCA-precipitable material to total label in the tissue. RESULTS: There was no significant difference in protein synthesis due to nicotine in the three sections of the brain at day 10. There was significant inhibition of DNA synthesis in the brain sections of pups as a function of age of nicotine injection compared with controls and the percent change varied with the age and section of the brain. The day 10 pups given [^{3}H]-thymidine at selected times post nicotine, indicated that DNA synthesis was still depressed at 4 hours, but less so than immediately after nicotine injection. Pretreatment with blocking agents had no affect on inhibition of DNA synthesis in day 10 pups. Also, 100% oxygen

had no affect. The 2 μg given intracisternally reduced DNA synthesis whereas the same dose given subcutaneously had no significant affect. When the dams were given 3 mg/kg nicotine at day 20 of gestation, DNA synthesis in the midbrain + brainstem and the cerebral cortex was inhibited. There was insufficient sample for the cerebellum. Possible adverse effects. Supplemental study. No worksheet. (Kishiyama and Gee, 12/31/02).

001 135209 Prokopczyk, G., J. D. Adams, E. J. La Voie and D. Hoffmann. "Effect of Snuff and Nicotine on DNA Methylation by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone." (Naylor Dana Institute for Disease Prevention, NY, publ. in *Carcinogenesis* 8 (10): 1395 - 1397 (1987)). Snuff extract (0.5 ml solution of an aqueous extract) was administered via gavage to 6 male F344 rats twice daily, five days/week, and once daily on weekends, for 2 weeks. After 2 weeks of nicotine treatment, all rats received a single NNK (0.4 mmole/kg b.w.) treatment by gavage. Rats were sacrificed 4 hours after NNK treatment and lung, livers, nasal mucosae and oral cavity tissues removed. DNA was isolated and the levels of O^6-methyl guanine and 7-methylguanine determined by hplc. For comparison, nicotine solution (0.002%) was administered in drinking water to 30 male for 2 weeks. After two weeks, animals were injected IV with NNK (0.4 mmole/kg b.w.). Before injection and at several intervals thereafter, 2-4 males were sacrificed, blood removed for pharmacokinetics and lungs, livers, nasal mucosae and oral cavity tissues excised for DNA analysis. Blood was analyzed for NNK and its butanol metabolite, NNAL. RESULTS: **Snuff** extract: The levels of 7-MeGua were higher in control animals given snuff extract in the DNA of the liver, nasal cavity and oral cavity but lower in the lung. O^6-MeGua could not be detected in the lung or oral tissue and was much higher in control animals in the liver and nasal cavity. For **nicotine** treated rats, the DNA methylation for the treated and control animals did not differ significantly in animals sacrificed at 0.5, 1.0, 4, 12 or 24 hours after NNK injection. The levels of methylation, however, increased during the 24 hours but began to drop off in the nasal tissue between 12 and 24 hours. Methylation was most extensive in the nasal mucosa followed by the liver, oral tissues and lung. Levels of NNK and NNAL in circulating blood were similar for nicotine treated and control animals with similar elimination rate constants. The results indicated that snuff extract altered the level of methylation of DNA while nicotine did not. Supplemental study. Adverse effect not identified. No worksheet. (Kishiyama and Gee, 12/31/02).

OTHER

001 135213 Bhagat, B. and M. W. Rana. "Antitumor Activity of Antiserum Nerve Growth Factor (anti-NGF)." (Publ. in *Proc. Soc. Exp. Biol. Med.* 138: 983 - 984 (1971)) Newborn CF-1 mice were immunosympathectomized with antiserum to nerve growth factor administered daily at 0.07, 0.10, 0.10, 0.12, and 0.15 ml during the first 5 days of life. Control mice received horse serum injections. Twenty-seven days after birth, all test animals were injected subcutaneously with 3 mg of benzo[a]pyrene (repeated at 30-day intervals to mice having no lesions). Three days after the first injection of BaP, half the mice given horse serum were injected subcutaneously with 0.5 mg/kg nicotine and the other half (immunosympathectomized) with an equivalent volume of saline. Mice were observed for 170 days after BaP injection and the first appearance of a tumor recorded. The first tumor appeared at day 27 in control mice but was delayed until day 90 in immunosympathectomized mice and day 100 with nicotine pretreatment. The incidence was also lower for these two groups. The average weight of tumors, however, was increased with nicotine treatment (8 g versus 4.5 g in controls and 3.1 in immuno-sympathectomized mice, indicating nicotine increased the rate of tumor growth. Supplemental study. No worksheet. (Kishiyama and Gee, 12/31/02).